MICROSTIMULATION OF LUMBOSACRAL SPINAL CORD-MAPPING

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I. Introduction

During this quarter we continued and expanded our studies begun earlier this year, in which we examined the complex motor movements of the cat's hindlimb generated by focal microstimulation of a single site in the L_6 or L_5 spinal cord. The hindlimb motor activity involved multiple joints and consisted of hindlimb lifting, extension, as well as abduction and adduction when specific sites in the spinal cord were stimulated. The motor activity was in general ipsilateral to the stimulus, although some movement of the contralateral hindlimb was seen with stimulation near the midline. Microstimulation of a single site in the spinal cord for a brief period (6 seconds) did not seem to trigger long duration repetitive motor activity. At times, however, the motor activity continued for several seconds following termination of the stimulus. The details of these experiments are described below.

During this quarter tracing studies examining the location distribution of efferent, afferent and interneurons which innervate the external and internal anal sphincters also continued. These studies will be presented at the upcoming Society for Neuroscience meeting. These studies have been described in previous progress reports.

Also during this quarter our manuscript summarizing our colon microstimulation experiments was revised and is now in press in Brain Research.

II. Complex Hindlimb Motor Activity Generated by Microstimulation of the Lumbar Spinal Cord.

A. Methods

These studies were designed to determine the various types of complex hindlimb

motion that can be elicited by microstimulation with a single microelectrode at specific sites in the L5 and L6 lumbar spinal cord. The methods used in these studies have been described in a previous progress report and are briefly summarized below together with specific modifications in experimental design used in these studies (see also Fig. 1).

Adult male cats anesthetized with pentobarbital 30 -35 mg/kg were rigidly suspended in a spinal frame. The hindlimbs were allowed to swing freely, while the spinal cord was stimulated at various sites (L_5 or L_6 segments of the lumbar spinal cord). The hindlimb motion elicited by microstimulation of the spinal cord was quantified by videotaping the hindlimb with two color video cameras. One camera positioned at the side of the hindlimb the other at the back. Reflective red dots marked the hip, knee and ankle joints (Figures 1 and 3). These dots allowed for off-line measurement of joint angles and distance of hindlimb motion. The two cameras were positioned to allow detection of movement in three planes. A video frame grabber coupled with a computer allowed video frames to be captured and a CAD (computer aided design) program aided in the measurement of changes in joint angle and distance traveled by the hindlimb during focal microstimulation. Stick-figures showing the changes in hindlimb position could be generated from the reflective marks of each video frame (Figures 3 and 7). The hindlimb motion for each run was correlated with histological data indicating the electrode position.

Stimulus pulses are generated by a computer controlled constant current stimulator. The stimulus parameters for most of the studies reported here are: negative first charge balanced pulses 0.2 msec duration, 40 Hz, 0 to 100 μA (Figure 2 bottom). In order to produce a smooth movement of the hindlimb the intensity of stimulation is slowly increased from 0 to 100 μA using a sine wave function with a 2 second period (0.5 Hz - see Figure 2 top). Three cycles are

delivered to each site. The electrodes are moved in 200 micron increments (dorsal to ventral) beginning at the surface of the spinal cord (Figure 1).

B. Results

Four general patterns of hindlimb motion can be elicited by microstimulation at specific sites in the L_5 and L_6 lumbar spinal cord. They include hindlimb flexion or lifting, extension of the hindlimb, and abduction or adduction Several variations in each of the four basic movements were seen. These variations occurred at different sites in the spinal cord and also at the same sites in different animals, and consisted of changes in intensity and distance of hindlimb movement (Figure 7). In some instances the return of the hindlimb to it's resting position required several seconds after termination of the stimulation; at other sites the limb returned to the rest position in less than a second.

Hindlimb lifting (flexion) involved several muscle groups and motion about three joints - the hip, knee and ankle joints. As shown in Figure 4 the response to a slow increase in stimulus intensity followed by a decrease in stimulation produced a smooth hindlimb lifting followed by a smooth return to resting position. A different degree of hindlimb lifting was often elicited at different depths within the L_6 spinal cord as shown in Figure 5. The response at depth 0.0 in figure 5 is the hindlimb at rest (no stimulation). The most dorsal sites (from 0.4 to approximately 2.0 mm) elicit varying degrees of hindlimb lift to the same stimulus (peak responses are shown in Figures 5 and 6). A back view of the limb showed little abduction or adduction at the more dorsal sites in the spinal cord. At deeper sites (3.6 to 5.2 mm) some hindlimb extension is elicited and is accompanied by hindlimb abduction.

Hindlimb extension also involved several joints - hip, knee and ankle joints. Extension

was usually elicited at deeper sites in the spinal cord (see Figure 6) and could vary in intensity depending on the particular site. Unlike hindlimb flexion (lifting) hindlimb extension was often accompanied by either abduction or adduction of the limb (see Figure 6 bottom panel).

Adduction and abduction of the hindlimb was usually elicited by microstimulation deep within the ventral horn. The magnitude of the hindlimb motion varied with location within the spinal cord (see Figure 5 and 6 bottom) and to some extent between animals at the same apparent locations.

Figures 8 and 9 summarizes the locations and distribution of several hundred stimulation sites in the L_5 and L_6 segments of the spinal cords of 5 animals. Flexion and extension are plotted on the left hemisection of each figure and adduction and abduction on the right. Notice that flexion or hindlimb lifting is elicited primarily in the dorsal spinal cord. These sites include the dorsal horn, parts of the dorsal columns and dorsal half of the ventral horn. Hindlimb extension as well as adduction and abduction are elicited most consistently deep in the ventral horn and ventral funiculus

Hindlimb movement contralateral to the site of microstimulation was seen only rarely and appeared as several rapid twitches. The twitches occurred while the ipsilateral hindlimb had three cycles of smooth lifting followed by relaxation (three cycles correspond to repeating the test stimulus three times at each site as shown in Figure 2).

C. Discussion

Focal microstimulation of specific sites in the L_5 and L_6 segments of the lumbar spinal cord elicited what appeared to be functional hindlimb movements. It should be emphasized that

these functional movements, although complex in nature and involving several joints and muscle groups, were elicited by microstimulation at a single site using a single electrode.

The hindlimb motor activity appeared functional in that the hindlimb was lifted with smooth excursion and returned to the resting position with a smooth downward motion. Although torque was not measured in these experiments the upward lifting movement produced considerable torque as estimated by manual palpation or restraint. The downward movement from the raised position was simply a slow release of the muscle contraction correlated with decreasing stimulus intensity and allowing gravity to return the hindlimb to it's resting position. The hindlimb extension elicited microstimulation in the ventral horn also appeared functional and produced considerable torque. In theory, by stimulating two sites on each side of the spinal cord movement resembling locomotion could be produced.

These studies will continue into the next quarter.

Figure 1. Schematic diagram of the experimental setup showing four main components of the preparation: (1) The cat hindlimb is innervated by several large somatomotor nerves which arise from the lumbosacral spinal cord. The major joints of the hindlimb – knee, hip, and ankle joints – are marked with red reflective dots. (2) The left hindlimb movement is recorded via two color video cameras connected to two videotape recorders (VCRs). One camera is aimed at the left side of the hindlimb while the second records movement from the back. The video frames were captured by computer using a video frame grabber and analyzed. (3) Two digital counters within the field of view of each camera were used to display run numbers that correlated with depth of the microelectrode and stimulus parameters. (4) A programmable stimulator generated the waveform, which was passed through a constant current isolator and presented to the spinal cord via a single fine tipped microelectrode. An LED array was turned on during stimulus presentation, which was in the field of view of the video cameras. Stimulus information was also delivered to a loud speaker to provide auditory cue to the investigators.

Figure 2. A schematic diagram of the stimulus waveform showing the pulse shape (bottom) and the sinewave modulation of the amplitude (top). Each pulse is a negative first, charge balanced pulse having a duration of T=0.2 msec (bottom: stimulus waveform). The amplitude (A) of the waveform varies from 0 to 100 μA and is modulated by a sinewave function. The frequency is 40Hz. Each site in the spinal cord is tested with a six-second stimulus, the sinewave modulation of the amplitude has a two-second period and three cycles are applied.

Figure 3. Schematic diagram of the cat hindlimb showing placement of markers for each joint (hip, knee, and ankle) which are recorded on videotape. A CAD (computer aided design) program is used to connect the markers on digitized frames of the video, thereby producing "stick figures" of the hindlimb positions prior to and during microstimulation of the spinal cord. The stick figures are used to show changes in hindlimb positions during focal microstimulation at various spinal cord sites.

Figure 4. A series of stick figures of the cat hindlimb (top) showing changes in joint angle and hindlimb position during focal microstimulation of the L_6 spinal cord with a single microelectrode. The sinewave modulation of the stimulus amplitude is indicated schematically at the bottom. A single two second stimulus period is shown. The stick figure at "0" time is the starting position with the hindlimb at rest. Notice the graded lifting of the hindlimb as the stimulus intensity is slowly increased from 0 to 100 μA, and then the gradual return of the hindlimb to resting position as the stimulus intensity is reduced from 100 to 0 μA. Stimulus is the same as in figure 2 except only one cycle is shown.

Figure 5. Two series of stick figures of the cat hindlimb showing the peak response to focal microstimulation at various depths along a single electrode track (#1) in the L6 spinal cord. The views from two cameras are shown – the left side and a back view. The numbers across the top of each set of stick figures are depths from the L6 spinal cord surface. The 0.0 mm depth in each is the resting (non-stimulated) control position. Stimulus is as shown in Figure 2. A transverse section of the L6 Spinal cord is shown at the left of the figure, showing several electrode tracks. Data from track #1 is shown in this figure. Notice that from 0.4 to 2.4 mm from surface a lifting of the hindlimb is elicited while at deeper sites in the ventral horn hindlimb extension is produced. Notice also that the view from the back of the hindlimb indicates little movement until a depth of 3.4 mm is reached; where some abduction of the left hindlimb is elicited.

Figure 6. Same as figure 5 except showing little or not response at the most dorsal sites in the spinal cord but hindlimb extension at the deeper sites. The view from the back of the hindlimb (bottom set of stick-figures) shows some abduction from about 2.0 – 3.6 mm than adduction at deeper sites in L6 spinal cord. Stimulation parameters are same as figure 5.

Figure 7. Stick-figure of the hindlimb showing various responses seen in the L6 and L5 spinal cord. Broken lines are the resting hindlimb positions seen in the left and back views of the leg. The thick solid line is the maximal response seen with cord microstimulation and the thinner solid line is an intermediate response at some sites. Symbols at the bottom of each group of stick figures will be used to show the location of each response type on a transverse template of the L6 and L5 spinal cord.

Figure 8. Transverse section of the L6 spinal cord showing the location and distribution of sties which elicited flexion or extension of the left hindlimb (left template). Abduction or adduction of the left hindlimb are shown on the right spinal cord template, using symbols shown below each section and described in figure 7. Notice that flexion or leg lifting is elicited most often at dorsal sites in the L6 spinal cord, although at times extending into the ventral horn. Hindlimb extension occur mostly deep in the ventral horn. Abduction and adduction of the hindlimb is elicited mostly in the ventral horn and is frequently accompanied by leg extension.

Figure 9. Same as figure 8 except showing the L5 level of the spinal cord.

Experimental Setup

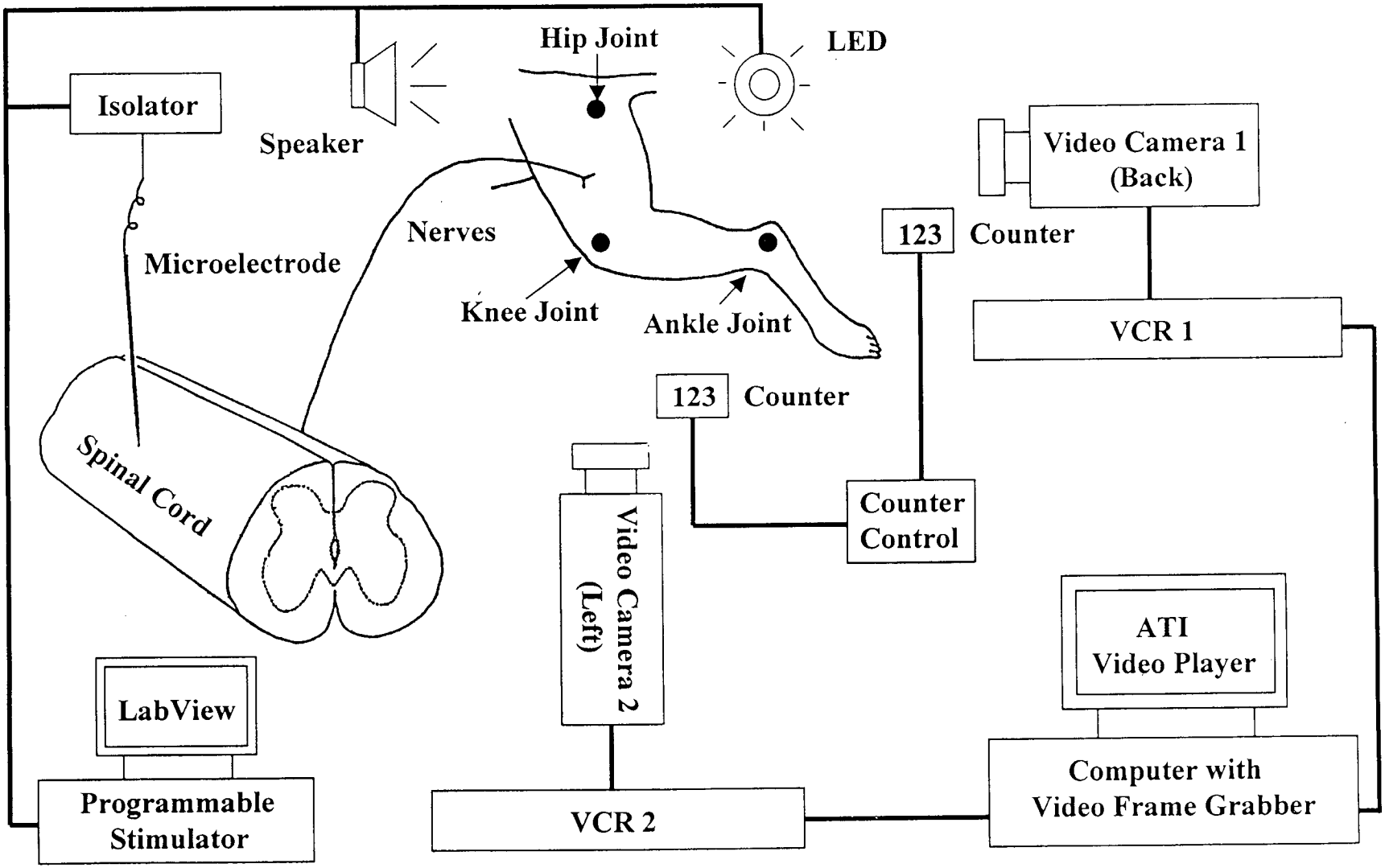
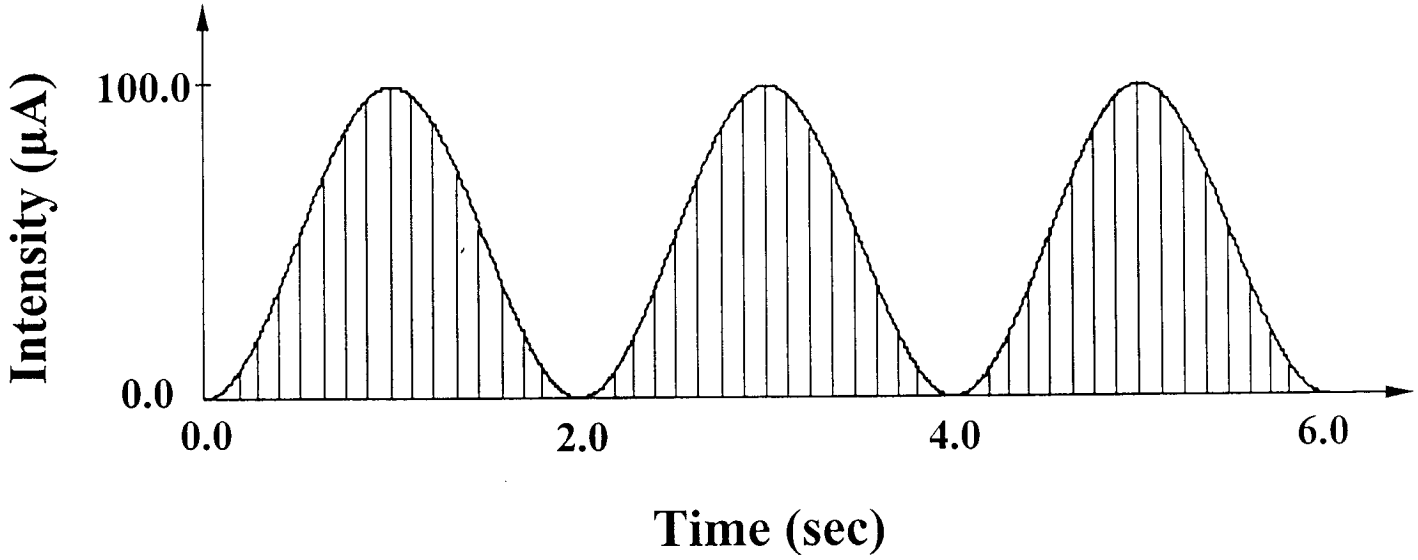


Figure 1

Microstimulation Waveform



Stimulation: Frequency = 40 Hz; Pulsewidth = 0.2 ms

Stimulus Waveform:

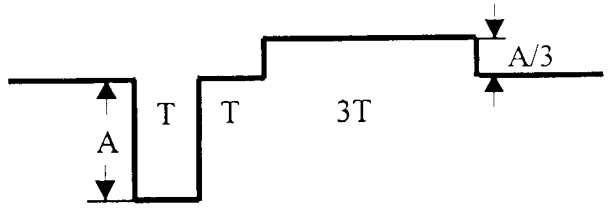


Figure 2

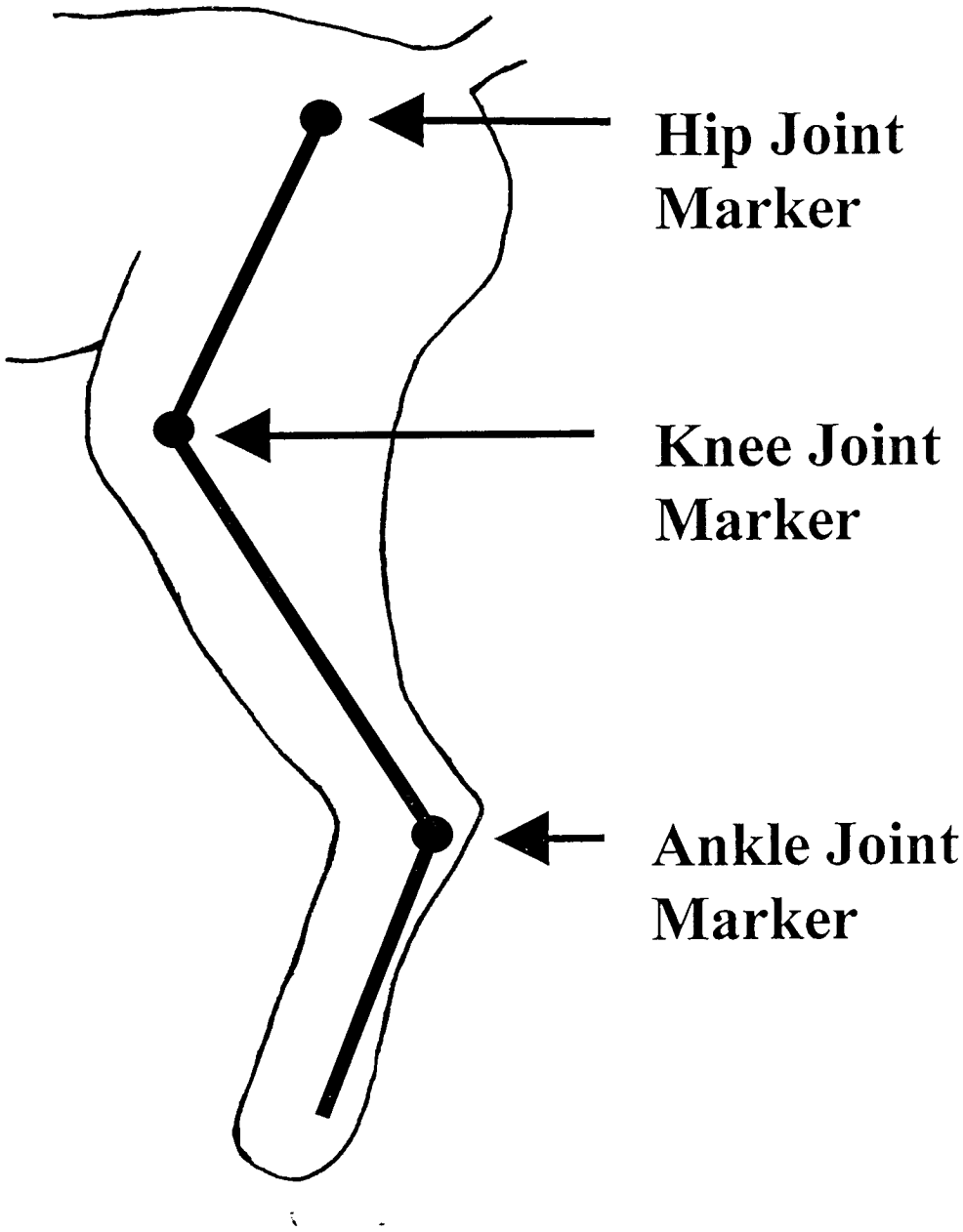


Figure 3

MS#77, Track#1, L6, depth 0.4mm, movement#1

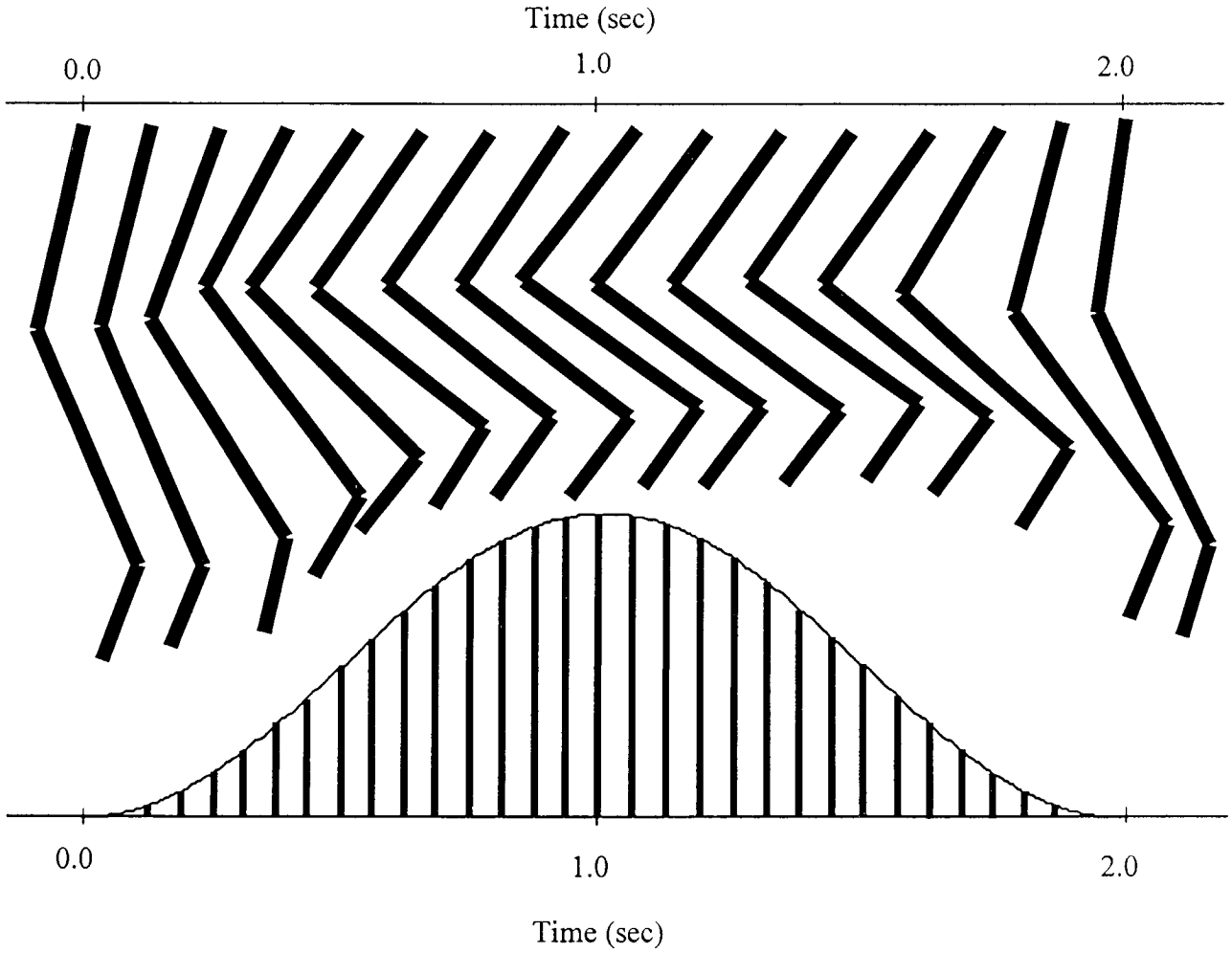
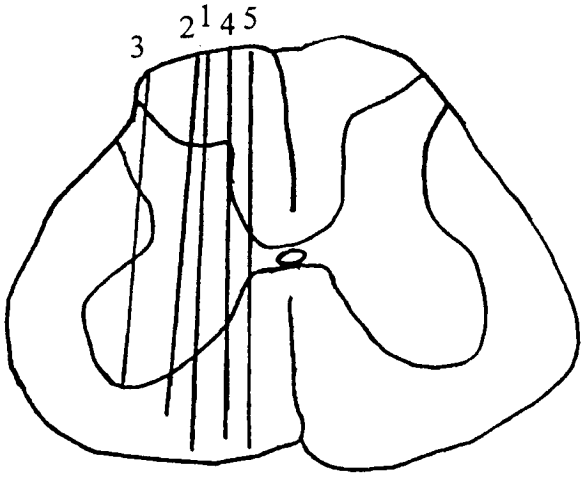


Figure 4

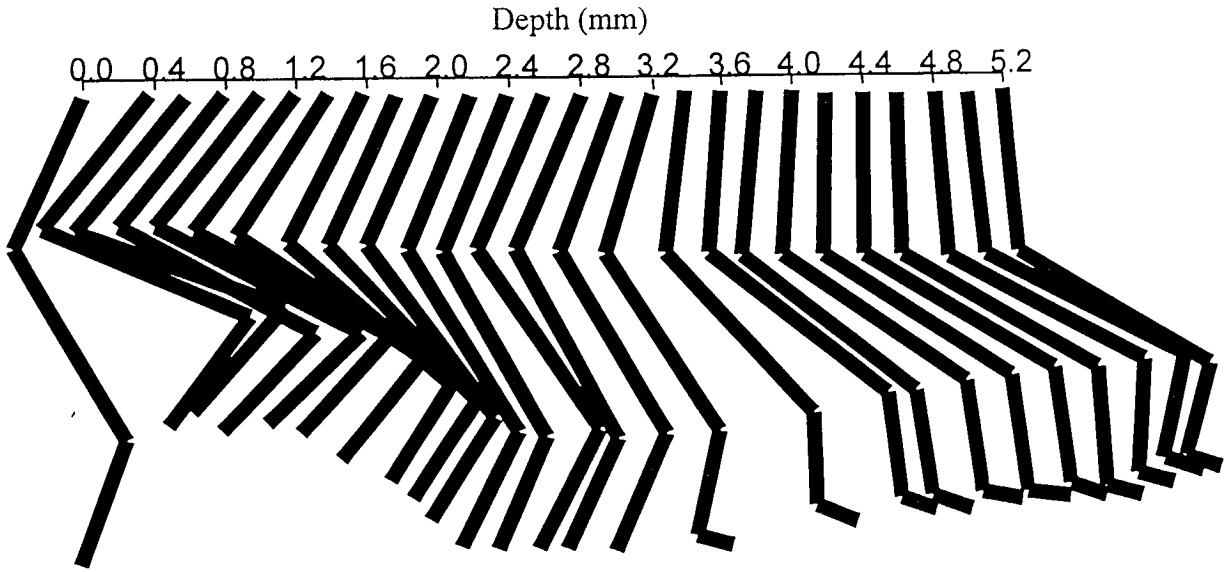
Microelectrode Track



L6 Spinal Cord

MS#80, Track#1,
Depth 0.4mm to 5.2mm,
No.1-5,7-26

View from Left Camera



View from Back Camera

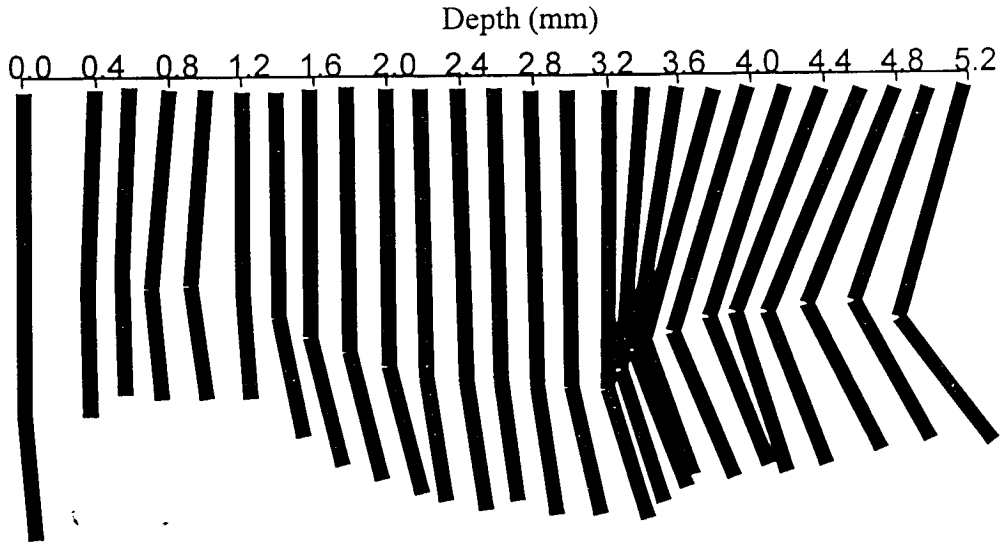
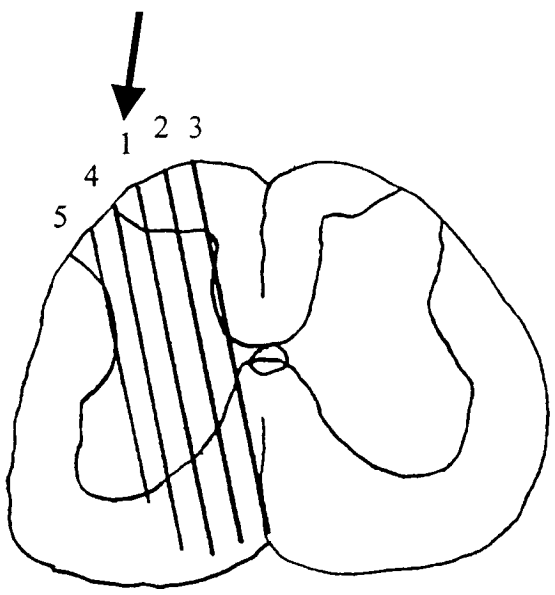


Figure 5

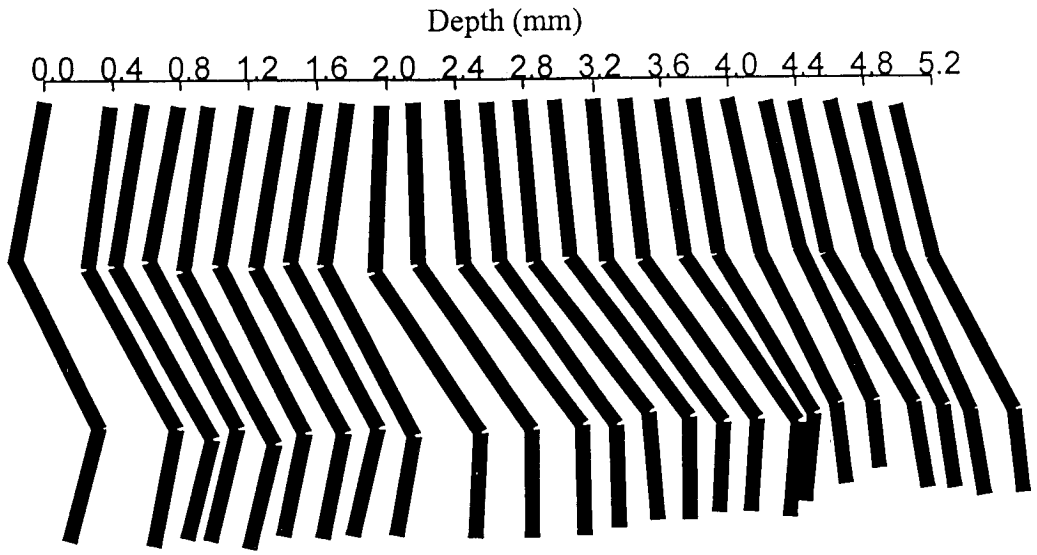
Microelectrode Track



L6 Spinal Cord

MS#78, Track#1,
Depth 0.4mm to 5.0mm,
No.1-24

View from Left Camera



View from Back Camera

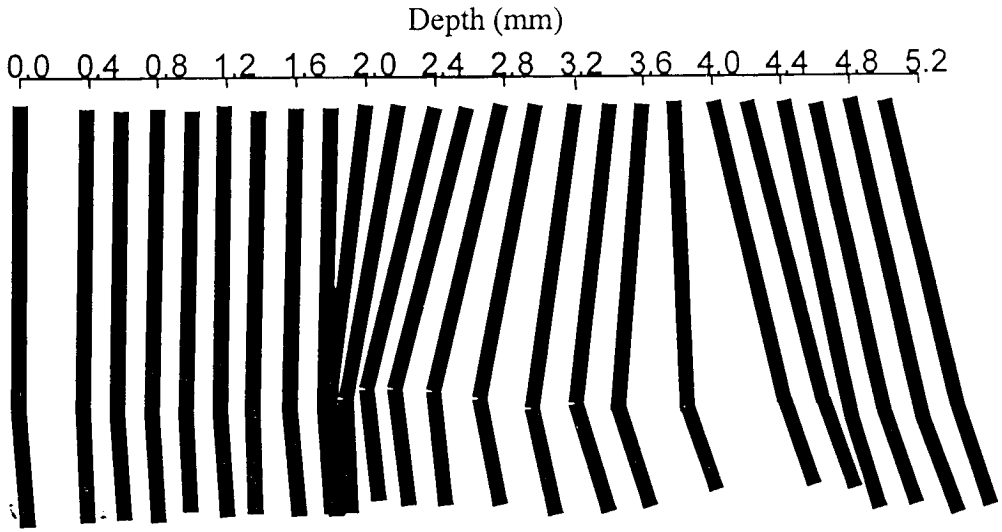


Figure 6

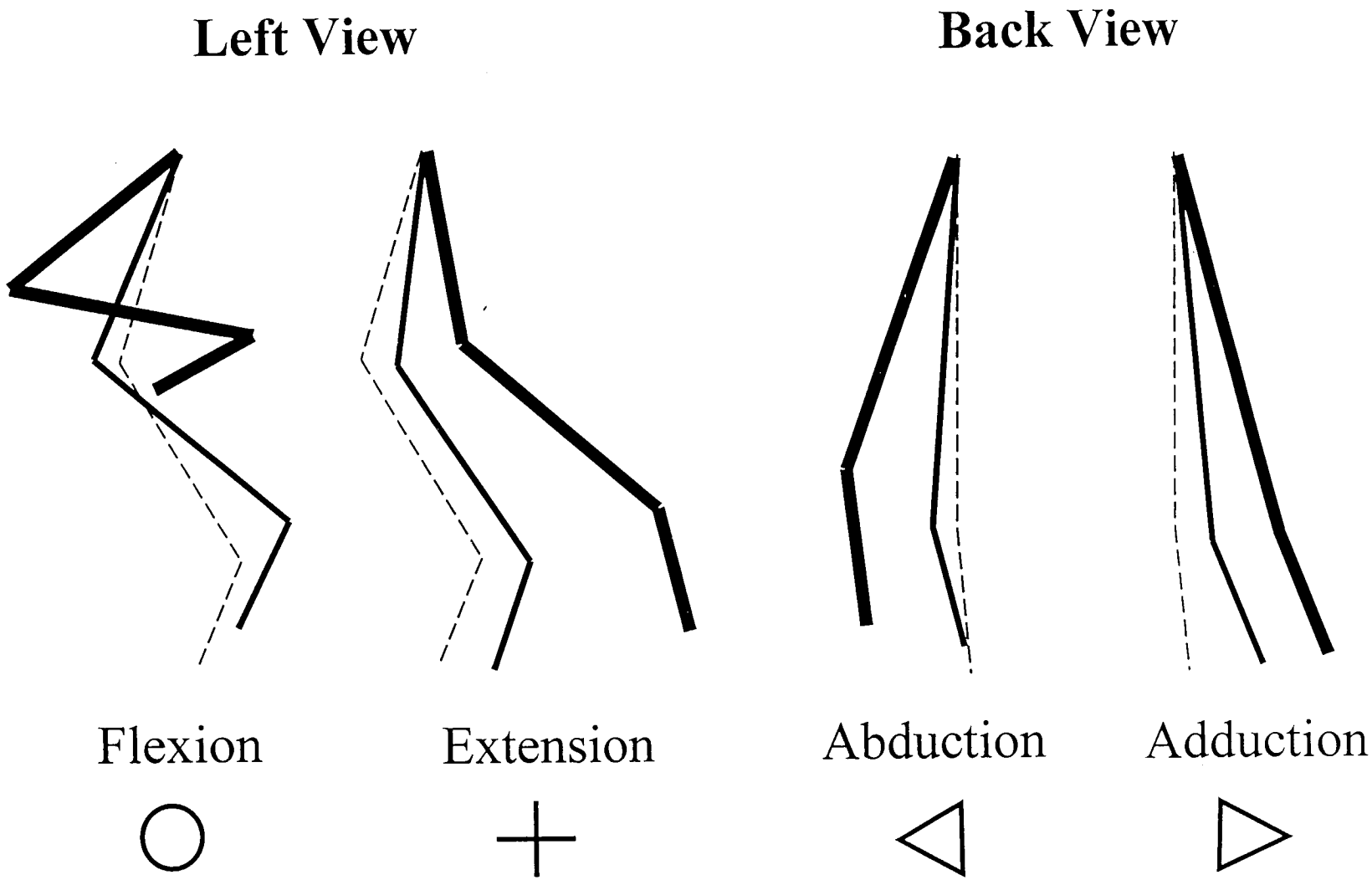

Left View
Back View
Flexion
Extension
Abduction
Adduction

Figure 7

Microstimulation Mapping of L6 Spinal Cord

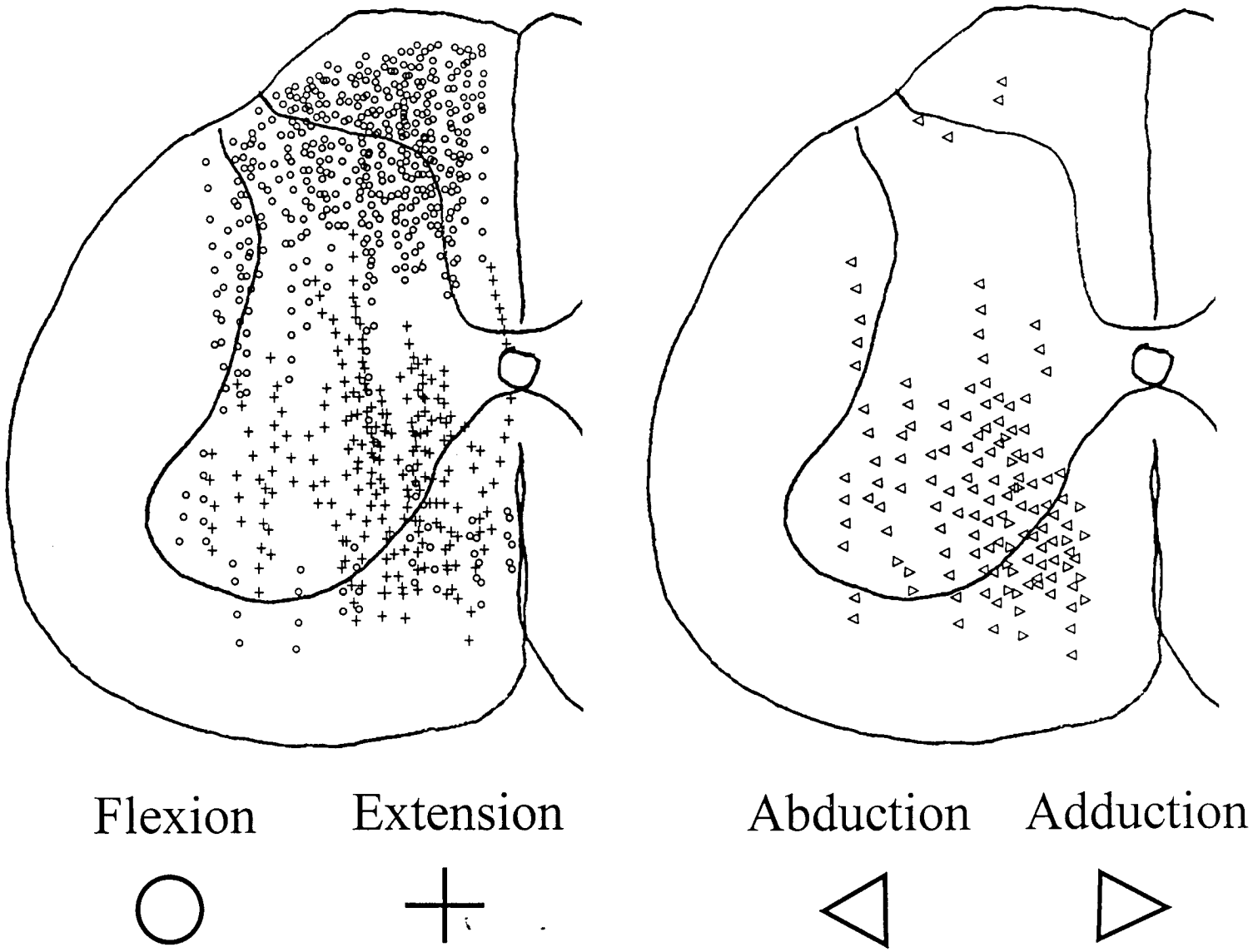


Figure 8

Microstimulation Mapping of L5 Spinal Cord

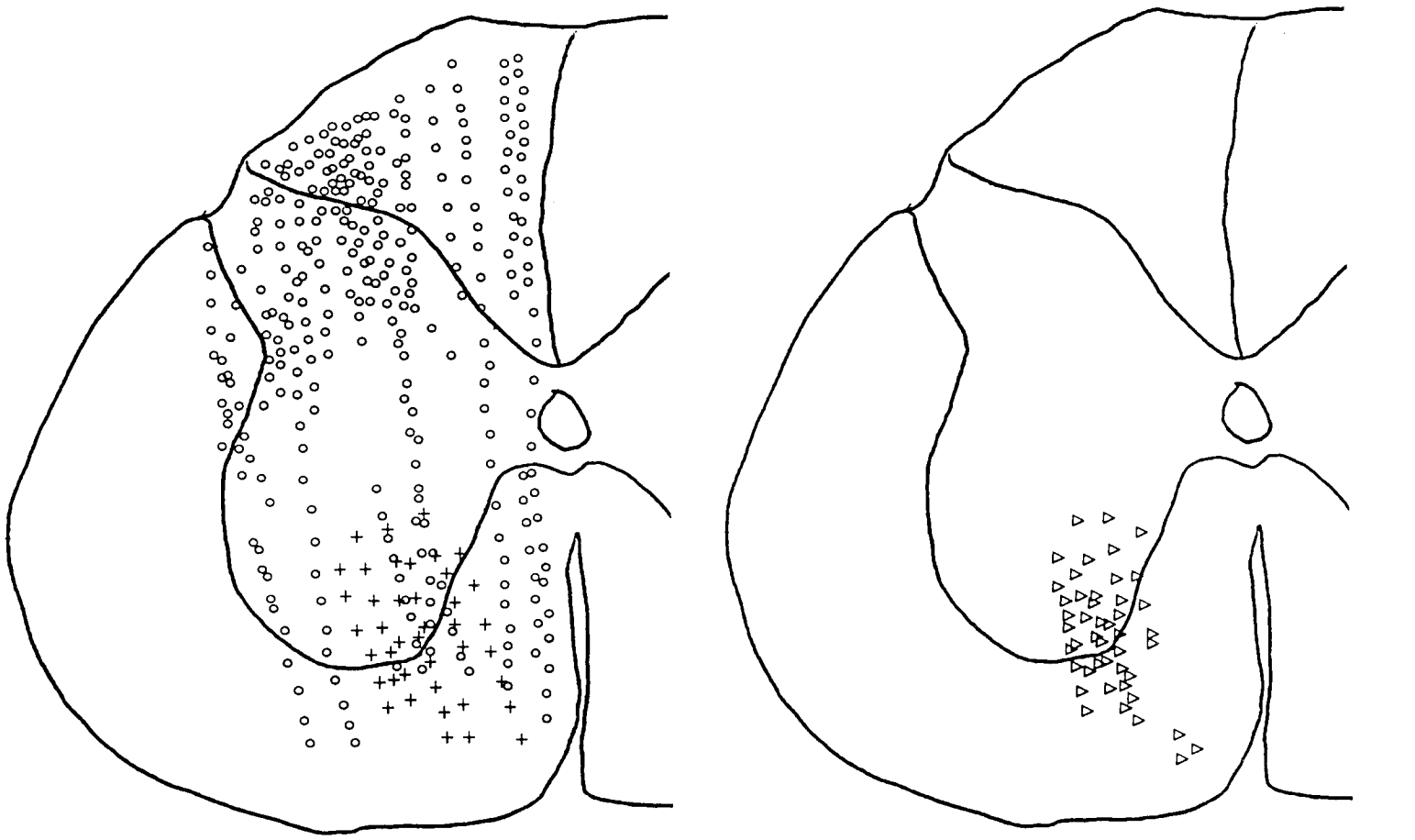


Figure 9